

BIOPROCESSING OF CRUDE OILS AND DESULFURIZATION USING ELECTRO-SPRAY REACTORS

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ABSTRACT

Biological removal of organic sulfur from petroleum feedstocks offers an attractive alternative to conventional thermochemical treatment due to the mild operating conditions afforded by the biocatalyst. Electro-spray bioreactors were investigated for use in desulfurization due to their reported operational cost savings relative to mechanically agitated reactors and their capability of forming emulsions $<5\ \mu\text{m}$. Here, the rates dibenzothiophene (DBT) oxidation to 2-hydroxybiphenyl (2-HBP) in hexadecane, by *Rhodococcus* sp. IGTS8 are compared in the two reactor systems. Desulfurization rates ranged from 1.0 and 5.0 mg 2-HBP/(dry g cells-h), independent of the reactor employed. The batch stirred reactor was capable of forming a very fine emulsion in the presence of the biocatalyst IGTS8, similar to that formed in the electro-spray reactors, presumably due to the fact that the biocatalyst produces its own surfactant. While electro-spray reactors did not prove to be advantageous for the IGTS8 desulfurization system, it may prove advantageous for systems which do not produce surface-active bioagents in addition to being mass transport limited.

KEY WORDS: oil desulfurization, *Rhodococcus*, electrostatic spraying, dibenzothiophene, biodesulfurization

INTRODUCTION

Biological refining of fossil fuel feedstocks offers an attractive alternative to conventional thermochemical treatment due to the mild operating conditions and greater reaction specificity afforded by the nature of biocatalysis. Efforts in microbial screening and development have identified microorganisms capable of petroleum desulfurization (see for example [1-12]), denitrification [6], demetalization [6], cracking [6, 13] and dewaxing. Further investigation and manipulation of enzymatic pathways responsible for these reactions [4, 14-17] has led to processes which are approaching commercial application, particularly in the area of biological desulfurization [7, 18]. Biological desulfurization of petroleum may occur either oxidatively (see for example [3, 5, 8, 9, 12, 17, 19-21]), or reductively [2, 22-24]. In the oxidative approach, organic sulfur is converted to sulfate and may be removed in process water. This route is attractive due to the fact that it would not require further processing of the sulfur and may be amenable for use at the well head where process water may then be reinjected. In the reductive desulfurization scheme, organic sulfur is converted into hydrogen sulfide which may then be catalytically converted into elemental sulfur, an approach of utility at the refinery. A sampling of desulfurization rates achieved with oxidative and reductive microorganisms have been summarized in [25]. Regardless of the mode of biodesulfurization, key factors affecting the economic viability of such processes are biocatalyst activity and cost, differential in product selling price, sale or disposal of co-products or wastes from the treatment process, and the capital and operating costs of unit operations in the treatment scheme.

The selection of the petroleum feedstock in biodesulfurization will play a large role in the overall economic viability of the process. Biodesulfurization may be utilized as a pretreatment to crude oil before entering pipelines, may be applied as an alternative to hydrotreating the crude at the refinery, or may be applied in the polishing of refinery products such as diesel or gasoline. The particular application will determine the extent of desulfurization necessary and hence the treatment cost per barrel. At the wellhead, a biodesulfurization unit may be used to treat marginally sour crudes (0.6 - 0.7% S) converting them to sweet crudes ($<0.5\%$ S) and claiming the price differential in sweet versus sour crude in segregated pipeline systems (currently, the premium for sweet crude is $\sim \$1/\text{Bbl}$). For this application, the extent of desired desulfurization is quite low and this may serve as an attractive initial niche for biodesulfurization. When utilized for refinery applications, the biodesulfurization process must compete

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with conventional hydrotreating. Here the economic viability of biorefining will be based upon its competitiveness relative to the catalyst replacement, hydrogen, and octane penalty costs associated with hydrotreating.

While significant activity is progressing in the engineering and chemical modification of enzymes so that they may function in purely organic solutions [26-28], inherent to all of the current bioprocessing of fossil feedstocks schemes is the need to contact a biocatalyst containing aqueous phase with an immiscible or partially miscible organic substrate. Factors such as liquid / liquid and gas / liquid mass transport, amenability for continuous operation and high throughput, capital and operating costs, as well as ability for biocatalyst recovery and emulsion breaking are significant issues in the selection of a reactor for aqueous / organic contacting. Traditionally, impeller-based stirred reactors are utilized for such mixing due to their ease of operation and wide acceptance in the chemical and biological processing industries. Such mechanically stirred reactors contact the aqueous and organic phases by imparting energy to the entire bulk solution, i.e. the impeller must move the contents of the reactor. Energy input in the stirred reactor is a function of the phase ratio, oil viscosity, density, reactor size, impeller speed, etc. [29]. Typically, impeller based reactors are capable of achieving water or oil droplet sizes of 100 -300 μm in diameter when surfactants are not present [25] and require on the order of 1-6 W/L to do so based upon empirical correlation's [29]. It is estimated that if impeller based systems were capable of producing 5 μm droplets, it would require ~25 kW/L [30] if surfactants are not present. Furthermore, no capacity exists for biocatalyst separation or emulsion breakage within the reactor.

Alternative processing schemes [18] propose the use of "motionless mixers", in which the two phases are pumped over a reversing helical coil which creates turbulent eddies. While this method reduces the number of moving parts in the reactor, it does not reduce power requirements since costs are transferred from the impeller to the pumps required to move the liquids past the coil. Liquid velocities greater than 4 m/s are required to form emulsions (~10 μm) and one can not form emulsions in coil tubes greater than 3 mm in diameter [31]. For tubes greater than 10 cm in diameter one can not form droplets smaller than 1 mm. Like the stirred reactor, no capacity exists for emulsion breaking within the motionless mixer.

Recent advances in the area of contactors for solvent extraction have lead to the development of electrically driven emulsion phase contactors (EPCTM) for efficient contact of immiscible phases [32-37]. In this concept, the differing electrical conductivity between the aqueous and organic phases causes electrical forces to be focused at the liquid / liquid interface, creating tremendous shear force (see for example [38]). This shear causes the conductive phase to be dispersed (5 μm droplet size - [30]) into the non-conductive phase, but does so with decreased energy requirements relative to mechanical agitators due to the fact that energy is imparted only at the liquid / liquid interface and not the entire bulk solution. Electrostatic crude oil desalters have been operated for several years [39]. More recently, devices based upon the EPCTM have been used commercially to water wash methyl tert-butyl ether feedstocks (with greater than 10-fold reduction in stage height [40]) and for organic extraction from aqueous analytical samples [41]. Energy consumption on a volumetric basis has been measured to be 2.4 W/L for a 30% tributyl phosphate / 70% dodecane / distilled water system, four orders of magnitude less than mechanical systems creating as fine an emulsion [30, 36].

The configuration of the EPCTM developed at the Oak Ridge National Laboratory is shown in Figure 1 where the contactor serves to disperse a liquid with a greater density than the continuous phase. The reactor employs two different types of electrode regions in order to increase liquid throughput. The first, termed the "nozzle region", provides a high capacity droplet dispersion by providing an electric field with a significant vertical component. This vertical field creates the dispersion at the nozzle entrance and accelerates it into the continuous phase. A second region termed the "operating channel" employs parallel plates carrying a modulated dc offset with high voltage spikes. This signal creates an oscillating horizontal electrical field which controls the residence time of the dispersed phase and serves to continuously coalesce and redisperse the droplets as they progress in a serpentine manner through the reactor. At the base of the reactor, an electrical field exists between the electrified central plate and the grounded aqueous phase, which accelerates the aqueous droplets to the organic / aqueous interface. In this manner, droplet coalescence and hence separation on the interface is enhanced. The EPCTM creates droplets of water containing biocatalyst ~5 μm in diameter within an organic phase, and does so with a power requirement of 3 W/L [25].

With the success the EPCTM has exhibited in the area of solvent extraction, it was proposed that it could be an efficient reactor system for aqueous / oil contacting in biorefining [42]. In our previous work [25], we characterized the emulsion quality and power requirements of the EPCTM, and demonstrated that there was no detrimental effect on the cells due to the electric fields. Here, we compare the performance of the EPCTM to that of a batch stirred reactor (BSR), investigate the required level of biocatalyst activity before the surface area afforded by the EPCTM becomes a factor in reactor performance, and characterize the emulsion formed by both reactors in the presence of bacteria.

MATERIALS AND METHODS

Biocatalyst and solvent systems

The oxidation of dibenzothiophene (DBT) in hexadecane was studied to investigate reactor design and performance in an easily tractable chemical system. *Rhodococcus sp.* wild strain IGTS8 (ATCC 53968) was provided by Energy BioSystems Corp. and served as the biocatalyst. The sequence of DBT oxidation by IGTS8 is shown in Figure 2, (Kilbane, 1989) and detailed enzymatic steps in the pathway are discussed in [17]. Cells were supplied as a frozen paste and had a cell dry weight of 0.28 g/g of original frozen material. The aqueous phase in all experiments consisted of 0.156M, pH 7.5 potassium phosphate buffer. DBT (Aldrich, D3,220-2), dissolved in n-hexadecane (Aldrich, H670-3), served as the organic phase, with typical initial DBT concentrations being 0.6 wt. %.

Analytical

Liquid samples collected from the reactors were centrifuged at 14,000 RPM for 5 minutes to separate the aqueous phase and cell debris. DBT and 2-hydroxybiphenyl (2-HBP) concentrations in n-hexadecane were measured by gas chromatography using a Hewlett Packard 5890 gas chromatograph equipped with a flame ionization detector. A 1 μ L sample was injected onto a 15 m DB-1 column (J&W Scientific, catalog number 125-1012) which was used with a helium carrier flow of 10 mL/min. The temperature program was 150°C for 1 min followed by an initial ramp rate of 5°C/min up to 200°C and a final ramp rate of 25°C/min to a final temperature of 280°C. The column was calibrated with DBT and 2-HBP (Aldrich, #24,021-4) standards. The injector was operated at 250°C and the detector was operated at 300°C. In the experiments reported here, DBT and 2-HBP concentrations in the aqueous phase were below our levels of detection. Hence only concentrations in the organic phase are reported.

Batch stirred reactor experiments

Experiments conducted in batch stirred reactors (BSR) typically utilized 50 g of frozen *Rhodococcus sp.* wild type strain IGTS8 (ATCC 53968) cell paste which were brought up to 750 mL with 0.156M (pH 7.5) phosphate buffer (1X cell density) and added to 250 mL of 0.6wt% DBT in n-hexadecane. The reactor vessel was a 1-L VirTis Omni-Culture fermentor (model 178657, Gardiner, NY), utilizing a 6-bladed Rushton-type impeller with 2 baffles. The impeller was mounted on the shaft 0.5 inches from the aerator and 2 inches from the bottom of the vessel. The reactor was kept at 30°C, agitated at 800 RPM, and aerated with either room air or pure oxygen at a rates of either 0.2 or 1.0 SLPM. Specific aeration procedures are noted in the text. To collect samples, agitation and aeration were ceased for 5 min to allow the aqueous and organic phases to separate. A 1.5 mL sample from the top of the organic phase was then taken for analysis.

Mass transport experiments were conducted in the BSR to determine if the desulfurization process was liquid-liquid (l-l) or gas-liquid (g-l) mass transfer limited. The mass transport experiments were conducted as above except that aeration was performed using 1/4 " stainless steel tubing rather than the aerator supplied by the manufacturer. Unlike the previous experiments, the mass transport experiments were organic continuous as 250 mL of aqueous phase was dispersed into 750 mL of hexadecane containing 0.6wt% DBT. Five batch stirred reactors were run simultaneously with different quantities of *Rhodococcus sp.* wild type strain IGTS8 (ATCC 53968) cell paste added to each reactor. Using a 250 mL basis for the aqueous phase, the quantities of frozen biocatalyst added to the 0.156 M (pH 7.5) phosphate buffer in each reactor were 8.3, 16.7, 83.3, 166.7, and 250 g respectively. The cell density corresponding to 16.7 g frozen biocatalyst in 250 mL aqueous phase was considered as 1X cell concentration. Thus, the cell density was varied from 0.5X to 15X in this experiment. To collect samples, agitation and aeration were ceased for 5 min to allow the aqueous and organic phases to separate. A 1.5 mL sample was taken from the top of the organic phase in each reactor every hour for 10 h, and again at 24 h into the experiment.

Gas - liquid mass transport experiments were conducted using air and oxygen at 0.2 and 1.0 slpm. In order to determine whether or not an oxygen mass transport limitation was a factor in the experiments using different cell densities, two batch stirred reactors were run at 800 RPM, 30°C, containing 750 mL hexadecane with 0.6 wt.% DBT, 166.7 g biocatalyst (10X cell density) in 250 mL of phosphate buffer. The aeration rate was 1.0 slpm of room air for the control condition, and 1.0 slpm pure oxygen in the experimental condition. Samples were collected every hour for 8 h using the procedure described above.

EPC™ experiments

The design and operation of the EPCTM utilized in these experiments are adaptations of those described by Scott *et al.* [36]. A schematic of EPCTM operation is shown in Figure 3. The Teflon body of the EPCTM measured 10 cm x 10 cm x 61 cm. The front and rear plates were made of clear Lexan, allowing for visual inspection of reactor operation. A thin sheet of Teflon was placed between the body and the front and rear plates to prevent wetting and current leakage to the Lexan. Three stainless steel electrodes, placed parallel to each other, measured 30 cm x 6 cm. The center electrode was charged, while the two outer electrodes were grounded. This adaptation to the original EPCTM design was found to minimize biomass fouling of the reactor. The center electrode was connected to the high-voltage electric supply through a supporting steel rod to avoid disturbance of electrostatic-spraying in the nozzle region. High-voltage (up to 40 kV) was generated using a pulsed DC power supply and automobile ignition parts. A power supply (Hewlett Packard 6653A, Avondale, PA) and two sweep/function generators (BK Precision 3030, Chicago, IL) were used to produce the signal which was then passed through an ignition coil (Mallory Promaster 29901, Carson City, NV) to step up the signal. In order to prevent discharge of the electrodes through the circuit, a high-voltage diode (Collmer Semiconductor CS4107X30, Dallas, TX) was placed between the coil and the EPCTM. From the diode, the positively charged terminal was connected to the rings surrounding the capillary tubes, and the negative lead was attached to the center electrode. The charged rings created an initial dispersion of biocatalyst as the aqueous phase emerged from the capillary tubes. The parallel electrodes lower in the reactor enhanced droplet dispersion as the more dense aqueous phase descended to the bottom of the reactor.

In EPCTM experiments, the organic liquid served as the continuous phase into which an aqueous biocatalyst was dispersed. The organic phase consisted of 2,400 mL n-hexadecane containing 0.6 wt.% dibenzothiophene. The temperature of the organic phase was controlled at 30°C by pumping the liquid from the top of the EPCTM, through a stainless steel coil submerged in a heated bath, and then returning the hexadecane to the bottom of the EPCTM. Typically, biocatalyst (26.7 g of frozen cell paste was brought to a volume of 100 mL with potassium phosphate buffer) was recirculated through the reactor at 5.0 mL/min using a peristaltic pump. The cell density with respect to the amount of aqueous phase used in these EPCTM experiments corresponded to a 4X concentration. A higher cell density was used in the EPCTM as compared to the BSR experiments due to the lower aqueous:organic phase ratio in the EPCTM and to enable DBT conversion significant enough to be observed by the analytical procedure employed. Aqueous phase containing the biocatalyst was sprayed into the reactor at the nozzle region, was continuously coalesced and redispersed in the operating region, and coalesced at the base of the EPCTM. To better aerate, sample, and control the aqueous phase, it was circulated from the reactor base to an external reservoir at a rate of 5.0 mL/min. The external container allowed for temperature control, pH and O₂ measurement, agitation, and aeration of the biocatalyst. The liquid was then returned to the top of the reactor through two 1.6-mm-OD, 1-mm-ID capillary tubes (U-140, Upchurch Scientific, Oak Harbor, WA) where it was again sprayed into the hexadecane. A water bath controlled the temperature of the jacketed aqueous reservoir at 30°C. The pH was monitored throughout the experiment, and remained in the pH range of 7.0 to 7.5. Agitation of the aqueous reservoir from a magnetic stirrer and room air aeration through a diffuser at 20 mL/min permitted more favorable conditions for the biocatalyst than did the long residence time at the bottom of the EPCTM, a location which lacked both aeration and agitation. In order to help alleviate possible oxygen deficiency inside the reactor, another diffuser was introduced 3 cm from the bottom of the EPCTM at airflow rate of 36 mL/min. Samples from the EPCTM were drawn hourly for twelve hours through a 2m x 3.175mm Teflon tube placed 10cm through the top of the reactor against a sidewall. Using a 30mL glass-bodied syringe, the tubing was flushed with liquid from the EPCTM three times before a 1.5mL sample was drawn. Samples were centrifuged as described above. Due to the small amount of 2-HBP production in the EPCTM, and hence greater associated error in GC analysis, samples were run in triplicate.

In experiments to determine possible mass transport limitations in the EPCTM, 66.7 g of frozen cell paste was brought to 100 mL with potassium phosphate buffer (10X cell density) and used as the aqueous phase. In order to evaluate if oxygen mass transfer was a limiting factor, the aqueous reservoir used for recirculating the aqueous phase was sparged with pure oxygen at a flow rate of 50 mL/min. This experiment was also conducted at a 10X cell density.

RESULTS AND DISCUSSION

2-HBP production in the EPCTM and BSR

Desulfurization activity of the *Rhodococcus sp.* in both reactors was typically between 1 and 5 mg 2-HBP produced per dry g of biocatalyst per hour. Rates of 2-HBP production in the two reactor systems were within experimental variance and no appreciable difference in desulfurization rates were seen between the two reactors. Note that in the experiments reported here, the only available carbon and energy source for the biocatalyst other than what may be carried over in the frozen cell paste, was

hexadecane and DBT. Other studies (outlined in [25]) have utilized additional external carbon and energy sources and have reported higher activities with *Rhodococcus* sp. Due to the high surface area reported in the EPCTM [25], higher rates were expected in the EPCTM, however, similar performance was observed in both reactors. Experiments were conducted with higher cell densities to determine at what point the BSR becomes mass transfer limited and the high surface area afforded by the EPCTM would become beneficial.

Mass transport limitations

Results of the DBT desulfurization experiments conducted at varying cell densities in BSR's are given in Figure 4. The rate of desulfurization, when normalized with respect to cell mass, was found to decrease with increasing cell density indicating that mass transfer resistance was the controlling process in desulfurization. A statistical analysis of the data was conducted using the analysis of variance (ANOVA) test and the t-test (Table 1). Based on a 95% confidence interval, a significant difference in the rates of HBP production was observed between 5X and 10X cell density BSR's. This suggests that the desulfurization process becomes mass transfer limited at a cell density of 10X. The mass transfer limitation may be due to gas-liquid or liquid-liquid mass transport resistance.

The results of experiments conducted in the BSR at 10X cell density to evaluate possible gas-liquid mass transfer limitations are given in Figure 5. Increasing the rate of air supply or increasing the oxygen tension in the reactor through the use of pure oxygen rather than air was not seen to affect HBP production. It was determined that oxygen mass transfer was not the limiting factor in the desulfurization of DBT even at 10X cell density. This suggests that the system may be limited by liquid-liquid mass transfer. Since the EPCTM reportedly provides larger liquid-liquid interfacial area, the BSR was compared with the EPCTM for desulfurization activity at equal cell density.

EPCTM vs BSR at 10X cell density

Comparison of the EPCTM and BSR at 10X cell density is given in Figure 6. As shown, no difference was observed in the desulfurization rates between the two reactors. Thus, either the system is not truly mass transport limited or the EPCTM does not provide a larger surface area for reaction under the present conditions.

Emulsion characterization

To determine whether the EPCTM offers larger surface area than BSR, samples were collected from the reactors and observed under a microscope using a 100x oil emersion objective. Due to the opaqueness rendered by high cell densities employed, observations could not be made *in situ* during reactor operation. Samples collected during the run were immediately mounted on a slide and observed under a microscope. In addition to sampling conducted during a run, samples were also collected at the end of a run from a cuff layer formed after gravity settling of the aqueous and organic phases. The cuff layer (which was previously characterized as being 92.7% hexadecane) was observed to contain a major portion of the biocatalyst. In addition, a significant amount of the biocatalyst was extracted and existed in the organic phase. Microscopic examination of samples from the cuff layer of the BSR showed a very fine emulsion with droplet sizes ranging from 1 to 10 μm . A similar cuff layer was also formed during EPCTM experiments. Average droplet size for EPCTM and BSR samples collected at 4 hours during normal operation were $2.54 \pm 2.40 \mu\text{m}$ and $3.08 \pm 1.78 \mu\text{m}$, respectively ($n > 300$). Figure 7 shows a micrograph of the emulsion obtained in an EPC. Thus, a very fine emulsion is formed in the EPCTM as well as the BSR, and it appears that it is for this reason that an augmentation in desulfurization rate is not seen in the EPCTM relative to the BSR. Formation of such an emulsion in the BSR may be presumed due to production of biosurfactants by the biocatalyst IGTS8. Other *Rhodococcus* sp. have been previously reported to produce glycolipids in the presence of hexadecane as well as some crude oils [43]. Earlier characterization of droplet size in a BSR [25] was performed either with no biocatalyst present, or with an extremely small amount to allow *in situ* observation of the droplets in the reactor. Our previous work revealed a decrease in droplet size from 210 to 118 μm when adding just 5 g of cells in 750 mL aqueous phase. Thus, the emulsion characterization reported here (with 67 g of cells in 250 mL aqueous phase) is in no way contradictory to our previous reports. Preliminary experiments varying the concentration of DBT ten-fold did not affect the rate of desulfurization, indicating that the aqueous side liquid mass transfer may be controlling.

CONCLUSIONS

The performance of EPCTM was similar to BSR for desulfurization of a model system containing DBT in hexadecane treated with biocatalyst *Rhodococcus rhodochrous* IGTS8. The system was not limited by

gas-liquid oxygen mass transfer at high cell densities (10X). The equal desulfurization rates in two reactors were due to almost equal interfacial area and the high degree of cell extraction into the organic phase. Higher interfacial area than normally expected in mechanically stirred reactors were realized in BSR, presumably due to formation of surfactants by the biocatalyst present. While EPC™ did not prove to be advantageous for the IGTS8 desulfurization system in terms of rates of desulfurization, it may prove advantageous for electro-static emulsion breaking, in reducing power requirements for mixing, and for creating large amounts of surface area for systems that do not produce surface active bioagents.

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Table 1. Results of analysis of variance (ANOVA) and t-Test (assuming unequal variance) comparing desulfurization rates over a range of cell densities. The second column gives p values for an analysis of variance over the complete range of data. The 3rd through 6th column gives p values for t-Tests comparing two experiments at a time. Numbers in parentheses indicate the cell densities compared. The p values comparing the data at 5X and 10X cell densities are all below 0.05, showing a significant difference between the two sets. This suggests that the desulfurization process may be mass-transfer limited at 10X cell density with statistical certainty.

Time, hr	ANOVA (p)	p (0.5 & 1)	p (1 & 5)	p (5 & 10)	p (0.5 & 5)
1hr	3.62E-10	0.2170	0.7420	0.0010	0.0739
2hr	4.53E-02	0.2150	0.0953	0.0177	0.1820
3hr	2.60E-03	0.0787	0.3380	0.0066	0.0600
4hr	5.40E-03	0.4706	0.3340	0.0006	0.0950
5hr	5.30E-03	0.3250	0.4600	0.0146	0.2190
6hr	3.89E-05	0.7440	0.3580	0.0370	0.1310
7hr	3.00E-03	0.0750	0.0384	0.0025	0.0390
8hr	2.00E-04	0.0328	0.0510	0.0190	0.0180
9hr	6.00E-05	0.0951	0.1984	0.0031	0.0110
10hr	1.18E-02	0.5820	0.1458	0.0066	0.1920
24hr	9.00E-07	0.1343	0.0301	0.0005	0.0110

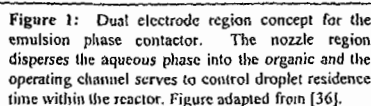


Figure 2: Biochemical pathway showing the sequence of DBT oxidation by IGTS8.

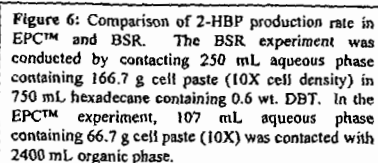
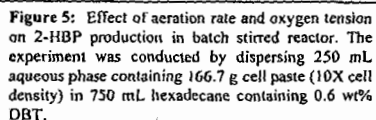
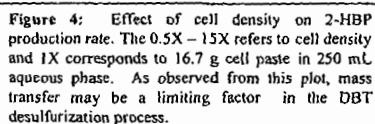
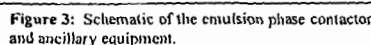


Figure 7: Micrograph of an EPC™ sample collected at 4 hours after beginning of reactor operation. The sample was collected from the central region between the electrodes using a Teflon tubing connected to a syringe and observed under a microscope as described earlier. The average droplet size was measured to be $2.54 \pm 2.40 \mu\text{m}$ ($n=300$).

